Supplemental Materials and Methods

Construction of rMboRNAP overexpression plasmid. The rMboRNAP overexpression plasmid was constructed in multiple steps. First, the *M. bovis BCG rpoA*, *rpoZ*, *rpoB* and *rpoC* genes were amplified from *M. bovis BCG* chromosomal DNA (prepared with generous help from Matyas Sandor, University of Wisconsin) using primers that added unique restriction sites (*Pme*I-*rpoA*-*Pac*I, *Pac*I-*rpoZ*-*Not*I, *Not*I-*rpoB*-*Asc*I, and *Nde*I-*rpoC*-*Asi*SI) and ligated into a pET21 (Novagen) derivative containing a reengineered polylinker of *Xba*I-*Pme*I-*Pac*I-*Not*I-*Asc*I-*Nde*I-*Asi*SI-*Xho*I restriction sites. Next, a linker encoding a flexible amino-acid sequence LARHGGSGA was used to connect the *rpoB* and *rpoC* reading frames. Finally, the His_8 tag was added to the 3′ end of *rpoC* to facilitate purification, resulting in plasmid pAC22. The *M. bovis sigA* gene was amplified from *M. bovis BCG* chromosomal DNA and cloned between *Nde*I and *Hin*dIII restriction sites of pRM629 plasmid, resulting in 5′-terminal fusion of *sigA* to a PreScission protease recognition site preceded by a His_{10} tag to give plasmid pAC27.

Construction of terminator template plasmids and templates. pRLG3748, which contains a -35/-10 consensus σ^{70} promoter followed by the *E. coli rrnB*T1 and T2 terminators 166 bp and 190 bp after the transcription start point (1), was used as the starting plasmid for construction of terminator assay plasmids. *Bam*HI and *Nco*I sites were introduced upstream and downstream from *rrnB*T1 by megaprimer mutagenesis with the oligonucleotide 5’-GTAGGGAACTGCCAGGATCCAAATAAAACGAAAGGCTCAGTCGAAAGACTGGGCCTTTCGTTTTATCTGTTGTTTGTCCATGGACGCTCTCCTGAGTAGG-3’. Terminators were then introduced into the resultant plasmid (pAC47; Table S1; Fig. S1) between the *Bam*HI and *Nco*I sites using annealed synthetic oligonucleotides with the desired sequences and requisite 5′overhangs (Fig. 2; Table S1). Subsets of the terminator plasmids were modified to encode an *Eco*RI site at 83-88 relative to the transcription start point to allow formation of halted ECs by *Eco*RIQ111 protein or to encode substitutions

of G, T, G, and G at positions +11, 14, 16, and 20 relative to the transcription start point to allow formation of halted U26 ECs when CTP was omitted (Table S1). Plasmids containing *Eco*RI sites at +83 also were altered by conversion of the *Eco*RI site at -60 relative to the transcription start point to CAGCTG to prevent effects of *Eco*RIQ111 on transcription initiation.

Purification of RNAPs. *E. coli* RNAP holoenzyme was purified from MRE600 cells as described previously (2). r*Mbo*RNAP was overexpressed from plasmid pAC22 in *E. coli* BL21 λDE3 pRARE2. RNAP overexpression was induced by addition of IPTG (250 μM final) to cultures at apparent OD_{600} 0.8, and incubation was continued for 4 h at 20 °C. Cells were chilled on ice, recovered by centrifugation (10,000 × *g*, 40 min, 4 °C), optionally stored as a cell pellet at -80 °C. Cell pellet (from 1 L culture) was resuspended in 30 ml 20 mM Tris-HCl pH 7.9, 5 mM imidazole, 500 mM NaCl, 5mM β-mercaptoethanol, 1 mM fresh PMSF, and supplemented with 1 ml of a solution containing 31.2 mg benzamide, 0.5 mg chymostatin, 0. 5 mg leupeptin, 0.1 mg pepstatin, 1 mg antipain, and 1 mg aprotinin. The suspended cell were lysed by French press at 8000-10,000 psi and 4 °C. The cell lysate was cleared by centrifugation (16,000 × *g*, 30 min, 4 °C) and passage through on 0.2 μ filter. The cleared lysate was bound to a 5-ml Ni^{2+}-NTA sepharose HP HiTrap column (GE Healthcare). The column was washed with 20 mM Tris-HCl pH 7.9, 5 mM imidazole, 500 mM NaCl, 5 mM β-mercaptoethanol until no A_{280} was detectable, and eluted with a 40 ml gradient of 5 to 1000 mM imidazole in 20 mM Tris-HCl pH 7.9, 500 mM NaCl, 5mM β-mercaptoethanol. The pooled fractions containing RNAP were dialyzed into 10 mM Tris-HCl pH 7.9; 200 mM NaCl, 0.1 mM EDTA, 1 mM $MgCl_2$, 20 μM ZnCl, 10 mM DTT, concentrated to 2 ml by centrifugation at 4 °C in a 15 ml Vivaspin device (10,000 MW cut off; GE Healthcare) according to the manufacturer's instructions, and separated on a 120 ml HiLoad Superdex 200 column (GE Healthcare). Fractions containing RNAP were pooled, concentrated to ≥1 mg RNAP/ml, dialyzed overnight at 4 °C into RNAP storage buffer (10 mM Tris-HCl

pH 7.9, 200 mM NaCl, 0.1 mM EDTA, 1 mM $MgCl_2$, 20 μM $ZnCl_2$, 50% glycerol, 2 mM DTT), and stored in aliquots at -80 °C.

M. bovis σ^A was expressed from pAC27 in *E. coli* BL21 λDE3 pRARE2. Cells were cultured in LB with shaking at 37 °C to apparent OD_{600} of 0.3, transferred to 30 °C until cultures reached OD_{600} of 0.6, induced for protein expression for 3 hours by addition of IPTG to 0.25 mM, and pelleted by centrifugation before storage at -80 °C (10,000 × *g*, 40 min, 4 °C). After cell lysis by French press, the cleared lysate was applied to a 5 ml Ni^{2+}-NTA sepharose HP HiTrap column as described above for the RNAP purification. Eluted samples were further purified by gel filtration on a HiLoad Superdex 200 column in 10 mM Tris-HCl pH 7.9, 300 mM NaCl, 0.1 mM EDTA, 1 mM $MgCl_2$, 20 μM $ZnCl_2$, and 1 mM DTT. The fractions were then pooled, concentrated, and dialyzed overnight at 4 °C into RNAP storage buffer, and stored in aliquots at -80 °C.

r*Mbo*RNAP holoenzyme was reconstituted by mixing purified core with 2- to 5-fold molar excess of *E. coli* σ^{70} or *M. bovis* σ^A and incubating at 37 °C for 30 min. Holoenzyme formation was confirmed by separation of the protein complexes by native gel electrophoresis (Novex NativePAGE Bis-Tris Gel) and staining with Imperial Protein Stain (Pierce).

Purification of *Eco*RI-Gln111. pVS9 (a generous gift of Dr. Irina Artsimovitch, Ohio State University) was used to express His_6-tagged *Eco*RIQ111 (3) in *E. coli* BL21 λDE3 at 37 °C. For cell lysis, pellets were resuspended in lysis buffer (40 mM Tris-HCl pH 7.5, 500 mM NaCl, 5 mM imidazole, 5% glycerol, 2 mM DTT, 0.1% Triton X-100) and sonicated at 4°C. Lysed cells were then centrifuged (10,000 × *g,* 40 min, 4 °C) and passed through an 0.2 μ filter. The lysate was added to Ni^{2+}-NTA agarose slurry (Qiagen) pre-equilibrated with lysis buffer and mixed gently at 4 °C for 1 h. The resin was then transferred to an empty 6 ml polypropylene column (Biorad) and washed with 10 volumes of lysis buffer supplemented with 50 mM imidazole. The purified *Eco*RIQ111 was eluted with the same buffer containing 150 mM imidazole. The purity in each fraction was assessed by SDS–PAGE/Coomassie staining and fractions containing only

the *Eco*RIQ111 protein were combined and dialyzed (10kDa cut-off cassette, Pierce) against *Eco*RI storage buffer (20 mM Tris-HCl pH 7.5, 300 mM NaCl, 50% glycerol, 10 mM β-mercaptoethanol, 100 μM EDTA, 0.15% Triton X-100) and quantified using a standard Bradford assay. The activity of purified *Eco*RIQ111 was verified by EMSA of DNA fragments containing *Eco*RI recognition sites.

Purification of NusA and NusG. *E. coli* NusA and *E. coli* NusG were purified as described previously (4, 5). Mycobacterial *nusA* and *nusG* were amplified from *M. bovis* chromosomal DNA and cloned into a plasmid (pRM629) containing the T7 RNAP promoter, N-terminal-His_{10} and PreScission-protease-site (LEVLFQ/GP) coding sequences followed by a polylinker. Digested PCR products were cloned between *Nde*I and *Hind*III sites in the polylinker to give pAC81 (NusA) and pAC82 (NusG). The proteins were partially purified using the protocol described above for *Eco*RIQ111 protein and then mixed with 0.04 mol equivalents of Precision protease (GE Healthcare), incubated at 4 °C 4 hours in 50 mM Tris-HCl pH 8.0, 150 mM NaCl, 5% glycerol, 1 mM DTT, 100 μM EDTA, 0.1% Triton X-100, and then passed over Ni^{2+}-NTA agarose to remove uncleaved protein. The tagless protein preparations were dialyzed into cleavage buffer supplemented with 50% glycerol, quantified by Bradford assay, and their identities verified by mass spectroscopy.

SUPPLEMENTAL REFERENCES

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